

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STATEMENT: BENZIMIDAZOLES: AN APPROACH TO DEFINING A COMMON ANEUGENIC GROUPING

COM/07/S3 – April 2007

Introduction

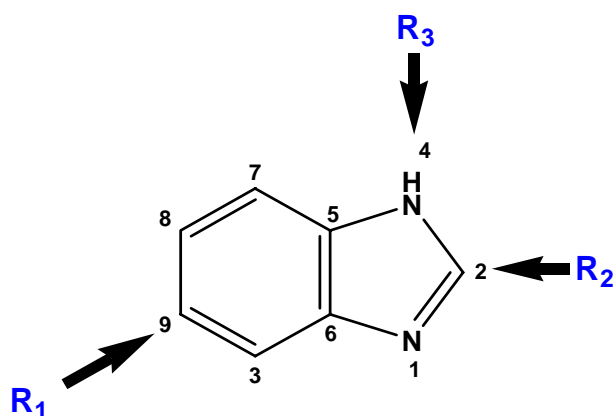
1. The COT published its report on Risk Assessment of Mixtures of Pesticides and Similar Substances in September 2002¹. One of the recommendations of the report was that a scientific and systematic framework should be established to decide when it is appropriate to carry out combined risk assessments of exposures to more than one pesticide and/or veterinary medicine. The COT recommended that the default assumptions in risk assessments of combined exposure should be that chemicals with different toxic action will act independently, and that those with the same toxic action will act additively¹. The COT has observed that where groups of chemicals with similar modes of action have been studied, additivity (or less than additivity) has been demonstrated rather than synergy. Examples include endocrine disrupters that act by agonism of oestrogen receptors, dioxin-like compounds and organophosphates^{1,2}.

2. Under dose additivity (also called simple similar action or simple joint action) the effect of a mixture is obtained by summing the doses of the individual compounds after adjustment for differences in their potencies¹. Because it occurs across the full dose-response curve, it is relevant to low doses. In contrast, if compounds have independent toxic actions, combined effects may be seen at high doses (e.g. due to effect addition or pharmacokinetic interactions), but would not be expected at doses below thresholds for the individual chemicals¹.

3. A new draft regulation proposed by the European Commission concerning the placing of plant protection products on the market (to replace Directive 91/414/EEC) requires additive and synergistic effects of pesticides to be taken into account in the approvals process when the methods to assess such effects are available³. The US EPA is already required by the 1996 Food Quality Protection Act in the USA to assess risks from exposures to combinations of pesticides.

4. One group of substances highlighted in the 2002 COT report as requiring further consideration as a possible common mechanism group was the benzimidazoles. These are substances used as pesticides (fungicides) and/or as veterinary medicines (mostly anthelmintics), which contain the benzimidazole ring (Figure 1). Additionally some pesticides and veterinary medicines are pro-benzimidazoles, i.e. they do not contain the benzimidazole ring but are metabolised *in vivo* to benzimidazoles. These compounds are considered here together with the benzimidazoles.

Figure 1: Molecular structure of the benzimidazole ring



5. The mechanism of action of benzimidazole compounds as fungicides is widely considered to be binding to free tubulin, particularly β -tubulin at the colchicine binding site, disrupting microtubule formation and thereby inhibiting mitosis^{4,5,6,7}. The primary mechanism of action of benzimidazoles as anthelmintics is also considered to be by binding to free β -tubulin and inhibiting its polymerisation^{8,9}, as a result affecting microtubule-dependent glucose uptake^{10,11}. A number of benzimidazoles have been shown to also inhibit mammalian tubulin polymerisation and to be aneugenic *in vivo*.

Definition of “common mechanism” of toxicity

6. The COT report did not specifically define “common mechanism” in its 2002 report. However, it variously referred to chemicals which would be in a common mechanism group as “similarly acting”, having “the same” toxic action, or having a “common mode of action”¹. The US Environmental Protection Agency (EPA), which is required by the 1996 Food Quality Protection Act in the USA to assess risks from exposure to combinations of pesticides, has defined a common mechanism group as consisting of “Two or more chemicals or other substances that cause a common toxic effect(s) by the same, or essentially the same, sequence of major biochemical events (i.e., interpreted as mode of action).”

(http://www.epa.gov/pesticides/glossary/index.html#common_mechanism, accessed 27 November 2006).

7. For the purposes of this review, aneugenicity was considered to be the toxic effect that the substances may have in common, as a result of inhibition of tubulin polymerisation.

Risk assessment of individual benzimidazoles

8. The COM has previously advised that it is reasonable to assume that aneuploidy-inducing chemicals (particularly those that function by damaging the cell division apparatus) have a threshold of action¹². In 1993 the COM

provided advice on methodology for identifying thresholds for aneugens acting by spindle inhibition¹². In 1996, the Committee considered the results of experiments undertaken with the benzimidazoles benomyl and carbendazim and concluded that the studies had been satisfactorily conducted and the data indicated No Observed Effect Levels (NOELs) for these two chemicals¹³. The Committee also saw similar data for thiophanate-methyl¹⁴. The Committee advised in 2000 that there is a sound scientific basis to assume that these chemicals have a threshold of action in both somatic and germ cells¹⁵. Other committees have drawn similar conclusions for other benzimidazoles where genotoxicity data have provided evidence for aneugenicity.

Review of the data on benzimidazoles

9. The Committee considered a review of findings in genotoxicity studies and other data relevant to assessments of aneugenicity, both from the regulatory assessments of authorised benzimidazoles (and pro-benzimidazoles) and from additional relevant papers identified in the published peer-reviewed literature. Since this was a consideration of a general approach to grouping these compounds, the Committee was not asked to evaluate the individual compounds. The findings are summarised in Table 1.

10. The data indicate that helminth tubulin binding is a relatively poor predictor of *in vivo* aneugenicity, and the Committee considered that aneuploidy in fungi could also not be considered to be a reliable predictor of *in vivo* aneugenicity in mammals. However, there were good correlations for these compounds between inhibition of mammalian tubulin polymerisation and evidence for *in vitro* aneugenicity in mammalian cells, and between *in vitro* aneugenicity in mammalian cells and *in vivo* aneugenicity for these compounds.

11. Results in the mouse lymphoma assay did not appear to be a good predictor of results in the *in vivo* micronucleus assay (data not shown in Table 1). For 4 compounds which were positive in an *in vivo* micronucleus assay and were studied in the mouse lymphoma assay, two were negative in the mouse lymphoma assay, one was clearly positive, and one was equivocal as there was not a clear dose-response relationship.

Additional relevant data

12. Two non-benzimidazole substances which both reduce tubulin polymerisation but by binding to different sites of tubulin (dilanatin and

Table 1: Summary of positive and negative results indicating action on tubulin and *in vitro* and *in vivo* aneugenicity. Note that some of the results are from studies reported in the peer-reviewed literature, which have not been critically assessed by this Committee.

Chemical	Binding to helminth tubulin	Inhibition of mammalian tubulin polymerisation <i>in vitro</i>	Aneugenicity in non-mammalian cells [†]	<i>In vitro</i> aneugenicity in mammalian cells [‡]	<i>In vivo</i> aneugenicity (e.g. bone marrow micronucleus assay)
Albendazole ^a	Positive	Positive	ND	Positive in micronucleus assay. No kinetochore staining	Positive in bone marrow micronucleus assay. No kinetochore staining
Albendazole oxide ^a	Positive	ND	Positive	Positive in micronucleus assay. No kinetochore staining	Positive in bone marrow micronucleus assay. No kinetochore staining
Benomyl ^b	ND	Positive	Positive	Positive	Positive
Carbendazim ^b	ND	Positive	Positive	Positive	Positive
Febantel ^c	ND	ND	ND	ND	Negative
Fenbendazole ^c	Positive	ND	ND	ND	Negative
Flubendazole	Positive	Positive	ND	Polyploidy and cell transformation	Negative
Fuberidazole	ND	ND	ND	Inhibition of mitosis	Negative
Mebendazole	Positive	Positive	Positive	Positive	Positive
Netobimin ^a	ND	ND	ND	ND	Positive in bone marrow micronucleus assay. No kinetochore staining)
Omeprazole	ND	ND	ND	Positive in micronucleus assay. No kinetochore staining	Positive in micronucleus study in hepatocytes. No kinetochore staining
Oxfendazole ^c	Positive	Positive	ND	ND	ND

Oxibendazole	Positive	Positive	ND	Polyploidy and metaphases of abnormal morphology	Negative
Thiabendazole	Positive	Positive	Positive	Positive	Positive
Thiophanate methyl ^b	ND	ND	ND	ND	Positive
Triclabendazole	No	ND	ND	ND	Negative

ND: No data identified

^aNetobimin is metabolised to albendazole which is metabolised to albendazole oxide

^bBenomyl and thiophanate-methyl are metabolised to carbendazim

^cFebantel is metabolised to fenbendazole, which is metabolically interconvertible with oxfendazole

[†]Tests for mitotic aneuploidy in yeast and *Aspergillus nidulans*

[‡]Includes studies in human lymphocytes, human/mouse hybrid cell line R3-5, human ovarian granulosa cells, Chinese hamster LUC2 and DON:Wg3h cells, Chinese hamster primary cells, rat primary hepatocytes, with additional data from CHO cells and mouse embryo fibroblast C3H/10T1/2 clone 8 cells.

vinblastine) were reported to have dose-additive inhibitory effects on mammalian microtubule assembly when tested in combination¹⁶. In contrast possible synergy in the antiproliferative effects on mammalian cells was reported for two compounds which have different effects on tubulin (paclitaxel and vinorelbine), one reducing polymerisation and the other stabilising polymerised tubulin, preventing microtubule disassembly which is necessary for completion of cell division¹⁷. The Committee considered that evidence for a common functional effect of benzimidazoles on tubulin would be important for an assumption of dose additivity.

Decision tree for inclusion in a common mechanism group

13. The Committee considered that a decision tree would be useful to aid assessing inclusion of a benzimidazole in a common mechanism group. Key information required would include the chemical structure, results from *in vitro* studies in mammalian cells and/or *in vivo* micronucleus assays, and data to indicate that compounds act via a common functional effect on mammalian tubulin, i.e. inhibition of polymerisation (See Figure 2). It was not considered essential for a benzimidazole to have been studied *in vivo* for it to be included in the common mechanism group since the available data indicated that *in vitro* aneugenicity is a good predictor of *in vivo* aneugenicity for the benzimidazoles.

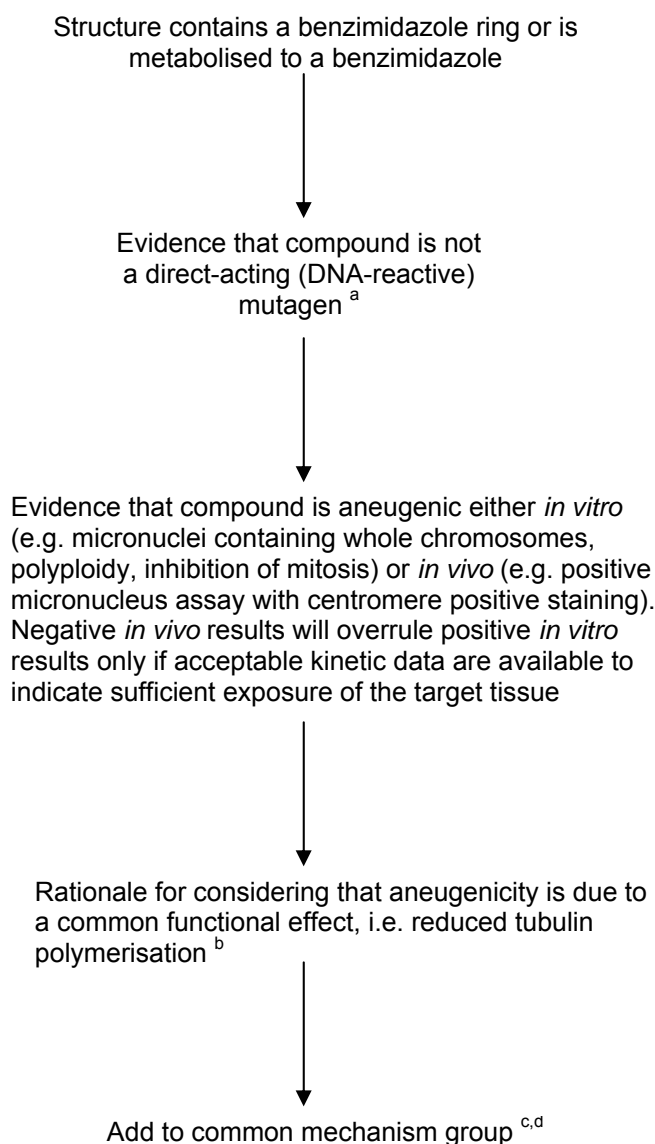
14. Since aneugens may also be clastogens, or may produce clastogenic metabolites, it was considered that genotoxicity data for benzimidazoles must be sufficient to demonstrate that mutagenic effects observed are solely due to aneugenicity, and this is reflected in the decision tree.

Research recommendations

15. The Committee recognised that an assumption of dose addition from combined exposure is pragmatic and is supported by data previously seen by the COT for compounds that act by various common modes of (non-genotoxic) action. However, in order to test the assumption and exclude the possibility of other, less-predictable, combined effects the COM recommended that several example pairs of benzimidazole compounds which pass through the decision tree be tested alone and in combination in the *in vitro* micronucleus assay.

16. We recognise that there is also a need to consider possible combined effects of benzimidazoles with other aneugens which interact with tubulin. We recommend that a suitable approach would be to test pairs of benzimidazoles and other aneugens which interact with tubulin in a similar manner as benzimidazoles (inhibition of tubulin polymerisation). In addition, any identified aneugens to which there may be co-exposure with benzimidazoles but which interact with tubulin in a different manner (e.g. by enhancing tubulin polymerisation) should also be tested in pairs with benzimidazoles.

Figure 2: Decision tree for including a compound in a benzimidazole common mechanism group for aneugenicity



Notes:

^a In some instances there may additionally be a need to assess risks from combined exposure with other benzimidazoles which are aneugens even if the compound may also be a direct acting mutagen, in which case the substance may continue through the procedure.

^b Any *in vitro* data showing reduced polymerisation of mammalian tubulin would be suitable

^c Mixtures of selected benzimidazoles assigned to the common mechanism group should be studied *in vitro* in order to test the default assumption of dose additivity and exclude the possibility of greater than additive effects (i.e. synergy).

^d Non-benzimidazoles which are considered to be aneugens may also be added to the common mechanism group if dose addition with at least one benzimidazole in the common mechanism group is demonstrated.

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